FURTHER STUDIES OF PLEOMORPHIC STREPTOCOCCI-BIOLOGIC REACTIONS

WITH ONE PLATE

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In a previous paper, one of us[1] described several pleomorphic strains of Streptococcus salivarius in tonsillitis, and in the course of routine work on meningococcus carriers, we made note of the relative frequency of pleomorphic streptococci and determined their classification. The organisms formed chains uniformly, under anaerobic conditions they remained small, and pleomorphism was constant under aerobic conditions. They had the same morphologic and cultural characteristics as many of the streptococci recently described by Rosenow and his associates[2] in poliomyelitis. In view of their interesting results we thought it worth while to compare the results of animal inoculations and immunological reactions. This seemed desirable as our results suggest an extensive distribution of the organisms. The cultural characteristics of the strains we studied are given in Table 1.

Probably the most satisfactory classification of streptococci that has been worked out so far is the one suggested by Holman[3] (Table 2). It is quite evident that the organisms we are interested in would be classified according to Holman as Streptococcus salivarius. They would also be classified the same according to Andrews and Horder's[4] classification, except that our strains are more or less pathogenic, whereas Andrews and Horder state that Streptococcus salivarius is nonpathogenic. Streptococcus anginosus is culturally the same as the salivarius except that it is hemolytic for several kinds of red blood cells.

We find that according to Holman's classification, Rosenow and his associates[2] have described 19 strains of S. salivarius, 21 strains of S. mitis, 6 strains of S. fecalis and several others that might or might not fit into Holman's classification, depending on capsule formation,

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[1] Sherwood, N. P.: Kans. Univ. Science Bull., 1917, 10, p. 247.
[2] Jour. Infect. Dis., 1918, 22, p. 313.
[3] Jour. Med. Research, 1916, 34, p. 377.
[4] Lancet, 1916, 2, p. 708.

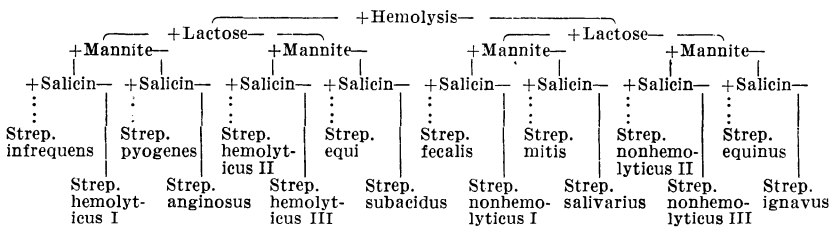
TABLE 1

NONHEMOLYTIC PLEOMORPHIC STREPTOCOCCI

Colony Type on Dextrose or Plain Blood-Agar Plates	Gram Stain	Gelatin Liquefaction	Litmus Milk		Fermentation Reaction Acid							Solubility in Bile	Capsule
			Acid	Acid Coagulation	Dextrose	Lactose	Mannite	Salicin	Raffinose	Saccharose	Inulin		
Small discrete colonies, brown by both reflected and transmitted light with a zone of brownish discoloration around each colony.	+	0	+	Variable	+	+	—	—	+	+	—	0	0

TABLE 2

STREPTOCOCCI-GRAM-POSITIVE COCCI IN CHAINS, NO CAPSULES



bile solubility and permanence of inulin fermentation. The decidedly pleomorphic strains as well as several at least of those used for animal inoculation, could be classified as S. salivarius. It has been our experience that practically all of the marked pleomorphic streptococci from the throat that show any degree of permanence of the tendency to produce involution forms, fall in this class.

In the course of a determination of meningococcus carriers we decided to note also the relative frequency of pleomorphic streptococci and to determine their classification and pathogenicity. We swabbed the nasopharynx of 130 schoolchildren and found pleomorphic streptococci present in 25%. During an epidemic of sore throat and pneumonia due to S. hemolyticus we found nonhemolytic pleomorphic streptococci in 19 of 78 throat cultures. Nasopharyngeal examinations for meningococci were made of 101 students and pleomorphic streptococci occurred in approximately 25%.

We used dextrose blood-agar plates. The medium was meat infusion agar with a reaction of 0.2% to phenolphthalein, and containing 2% dextrose and 5% whole blood. In view of the fact that dextrose interferes with hemolysis by some streptococci that are truly hemolytic, we carefully checked up this property on sugar-free blood agar. In determining the fermentations we found that sugar broths were not nearly so satisfactory as serum agar containing 2% of the desired carbohydrate and Andrade's indicator as used in the new Russell medium. In making up serum agar, we added 5 c c of serum, diluted 1:3 with distilled water and autoclaved, to each 100 c c of melted agar, and sterile carbohydrate solution in sufficient amount to make 2%, Andrade's indicator was added also. After thorough mixing the neck of the bottles was flamed and the medium poured directly into freshly sterilized test tubes, about 8 c c per tube. These were slanted as for Russell's medium and incubated to determine contamination. It was found advisable to make both surface and deep inoculation. Frequently acid production was noticed anaerobically but not aerobically. This is a satisfactory medium for pneumococci and also for quite delicately growing streptococci.

Twenty-four-hour cultures were used for animal inoculation. Rabbits weighing from 250-850 gm. mainly were used. The hair was clipped from the scalp and the animal put under light ether anesthesia. Working under sterile conditions the scalp was split longitudinally and drawn to one side. At first a small trephine was used, but later a hole was bored with a scalpel and the inoculation made through this opening. Inoculations of 0.25-0.5 c c of 24-hour cultures were made into the posterior part of the cerebrum in one set and in another set in the motor areas. Subdural inoculations were also made. In addition inoculations of 10-20 c c were made into the marginal ear vein. The weight and temperature were taken several times before the inoculations and at short intervals thereafter.

In all, 45 animals were inoculated; 29 with pleomorphic streptococci as follows: 8 intravenously, 18 intracerebrally in the posterior part of the cerebrum, 4 in the motor areas, and one subdurally. Six were inoculated intracerebrally with B. dysenteriae, B. typhosus and B. coli. In addition controls were inoculated intracerebrally and intravenously with sterile ascitic fluid dextrose broth and washings from sterile dextrose serum agar plates.

The results of our experiments are summarized in Table 3; the plus sign indicates positive and the minus sign negative results. It is realized that percentage figures in such a short series are not significant, but in view of the fact that others[2] in a similar series of animals used the percentage basis it seemed advisable for us to do so for purposes of comparison.

We see that the incubation period of the intravenously inoculated animals varied from 3-12 days, and that 50% showed a rise in temperature, tremor and loss of muscle tone. None, however, developed flaccid paralysis. Those inoculated intracerebrally had an incubation period of from 5-24 hours. Of these, 95.5% showed rise in temperature, 54.6% showed in addition loss of muscle tone, 4.55% developed flaccid paralysis, 22.75% showed rise in temperature only and 4.55% showed no change in temperature or other symptoms; 13.65%

TABLE 3

INJECTION OF RABBITS

Organism Injected	Method of Inoculation	Dosage in C C	Symptoms						Time before Symptoms Other Than Fever Develop	Remarks
			Rise in Temperature of 2-4 C	Tremor	Loss of Muscle Tone	General Weakness	Flacid Paralysis	Prostration from Overwhelming Inoculation		
Pleomorphic streptococcus	Intrav.	10.0	+	+	+	..	—	..	3 days	
Pleomorphic streptococcus	Intrav.	8.0	+	+	+	..	—	..	7 days	Died in 15 days
Pleomorphic streptococcus	Intrav.	10.0	+	+	+	..	—	—	7 days	Recovered
Pleomorphic streptococcus	Intrav.	10.0	+	+	+	..	—	—	8 days	Recovered
Pleomorphic streptococcus	Intrav.	10.0	+	—	—	+	—	..	.....	Died in 48 hours
Pleomorphic streptococcus	Intrav.	10.0	—	—	—	+	—	..	.....	Died in 4 days
Pleomorphic streptococcus	Intrav.	10.0	—	..	..	..	..	..	.....	Died
Pleomorphic streptococcus	Intrav.	10.0	+	—	—	—	—	—	12 days	
Controls (4)	Intrav.	10.0	—	..	—	—	—	..	.....	Remained normal
Pleomorphic streptococcus	Intrac.	0.5	+	+	+	—	—	—	12 hours	Progressive recovery
Pleomorphic streptococcus	Intrac.	0.25	+	—	—	. .	—	—		
Pleomorphic streptococcus	Intrac.	0.25	+	+	+	..	—	..	24 hours	Died in 48 hours
Pleomorphic streptococcus	Intrac.	0.5	+	—	+	..	—	—	5 hours	Recovered
Pleomorphic streptococcus	Intrac.	0.5	+	+	+	..	—	—	24 hours	Recovered
Pleomorphic streptococcus	Intrac.	0.5	+	—	—	..	—	—	.....	Recovered
Pleomorphic streptococcus	Intrac.	0.5	+	—	—	+	..	..	6 hours	Died in 48 hours
Pleomorphic streptococcus	Intrac.	0.5	+	—	..	..	..	+	.....	Died during night
Pleomorphic streptococcus	Intrac.	0.25	+	—	—	..	—	—	.....	Recovered
Pleomorphic streptococcus	Intrac.	0.25	+	+	+	..	—	—	5 hours	Recovered
Pleomorphic streptococcus	Intrac.	0.25	+	+	+	..	—	—	5 hours	Recovered after 6 weeks
Pleomorphic streptococcus	Intrac.	0.25	—	—	—	..	—	—	.....	Remained normal
Pleomorphic streptococcus	Intrac.	0.25	+	—	—	..	—	—	.....	Remained normal
Pleomorphic streptococcus	Intrac.	0.25	+	—	..	..	.	+	.....	Died during night
Pleomorphic streptococcus	Intrac.	0.5	+	—	..	..	..	+	.....	Died in convulsions after 24 hours
Pleomorphic streptococcus	Intrac.	0.5	+	—	—	..	—	—	.....	Recovered
Pleomorphic streptococcus	Intrac.	0.5	+	+	+	..	—	—	12 hours	Recovered
Pleomorphic streptococcus	Intrac.	0.9	+	..	+	..	+	..	5 hours	Died after 30 hours
Pleomorphic streptococcus	Intrac.	0.25	+	..	+	..	—	—	5 hours	Recovered
Pleomorphic streptococcus	Intrac.	0.5	+	..	+	..	—	..	5 hours	Died after 48 hours
Pleomorphic streptococcus	Intrac.	0.5	+	+	+	+	—	—	18 hours	Not completely recovered in 3 months
Pleomorphic streptococcus	Intrac.	0.5	+	+	+	—	..	—	18 hours	
Controls (4)	Intrac.		—	—	..	—	—	..	.....	Remained normal
B. dysenteriae	Intrac.	0.5	—	—	—	—	—	..	.....	Died in 18 hours, irritable marked toxic symptoms
B. dysenteriae	Intrac.	0.25	—	..	..	..	..	..	.....	Marked irritability, retraction of head, spastic paralysis of fore legs. Died in 12 hours (chloroformed)
B. typhosus	Intrac.	0.5	—	..	..	+	..	+	.....	Toxic symptoms, died in 15 hours
B. coli	Intrac.	0.5	—	..	..	..	..	+	.....	Died during night
B. typhosus	Intrac.	0.25	—	—	—	—	—	—	.....	Remained normal
B. coli	Intrac.	0.25	—	..	..	..	..	..	.....	Increased irritability and recovery

died within 24 hours from overwhelming infection. Of the animals inoculated with dysentery, typhoid, and colon bacilli, all showed subnormal temperature and marked increase in irritability with occasional spastic paralysis. This is not in harmony with Bull's results, who noted poliomyelitis-like symptoms after intracerebral inoculation of B. dysenteriae in rabbits.

ANATOMIC AND BACTERIOLOGIC EXAMINATION

Anatomic.—There were no marked changes observed in the body cavities of rabbits dying after either intravenous or intracerebral inoculations. The spleen was rather small and gray. Occasionally a few small areas of bronchopneumonia were noticed. Microscopically there were usually some fatty changes in the liver. The nervous system showed the most marked changes. Following intravenous inoculation none of the rabbits showed any evidence of purulent meningitis. The meninges were injected but there was no marked increase in fluid. There was apparently an increase in neuroglia and round cells in both the brain and cord. Figure 8 shows an apparent increase in round cells in the anterior part of the brain. After careful comparison with normal tissues there did not seem to be much perivascular infiltration of round cells in the intravenously inoculated animals, at least nothing like what was observed after intracerebral inoculation. There was, however, a noticeable increase in round cells and neuroglia cells with apparent degeneration of ganglion cells. In the intracerebrally inoculated animals there was always, as expected, a marked cellular reaction about the point of inoculation. Some polymorphonuclear leukocytes were present but the preponderance of cells were either neuroglia or round cells. This increase in round and neuroglia cells was not, however, confined to the point of inoculation; there were always areas of marked infiltration elsewhere in the brain also. Perivascular infiltration with round cells and hemorrhages were also present at other points in the brain and occasionally in the cord, decreasing downward. In one of the rabbits there was a marked meningeal exudate, and two hemorrhagic areas were found in the center of the cord.

Bacteriologic.—Blood-agar plate cultures from the heart blood and various organs were supplemented by taking one or more pieces of tissue from each organ and dropping them into dextrose broth tubes and incubating at 37 C. for several days.

If the animal died within 24 hours after intravenous inoculation the organisms were obtained always in pure culture from heart blood, various organs, and from the brain and cord. In 3 rabbits examined 4 days after inoculation, the heart blood and organs were sterile but the brain and cord yielded pure cultures of the organisms. In a third rabbit that died 4 days after inoculation the heart blood and organ cultures from liver and spleen were sterile but a piece of heart tissue after 3 days' incubation in dextrose broth, yielded a pure culture of the organism injected. From the brain and cord of this rabbit pure cultures were obtained. In this animal the heart blood drawn just before death as well as after death, gave no growth in dextrose broth. In stained sections of the nervous system of these rabbits streptococci were demonstrated.

In the intracerebrally inoculated animals the heart blood and organ cultures were uniformly negative but pure cultures were obtained from the brain and various parts of the cord. These animals had all been inoculated in the posterior part of the cerebrum.

IMMUNOLOGICAL

We have undertaken to find answers for the following questions:

Are there agglutinins for Streptococcus salivarius and mitis in the blood streams of supposedly normal individuals?

Are there normal complement fixing bodies in the blood stream of supposedly normal individuals?

Do inoculated rabbits develop agglutinins for any of these pleomorphic streptococci?

Are these pleomorphic streptococci agglutinated by serum that Rosenow obtained by immunizing horses against pleomorphic streptococci?

We found that growth from blood-agar plates was too dry and coherent to give a satisfactory emulsion when washed off in salt solution. Fresh serum dextrose agar plates yielded a more moist growth that made a uniform emulsion. Some clumps might be present, but these would settle out by standing. The most satisfactory method was growing the organisms in calcium carbonate broth and preparing suspensions from this growth. The agglutination tests were quantitative as well as qualitative and each tube in the series was made to contain 0.5 c c of suspension and 0.5 c c of diluted serum. This would give an ultimate dilution twice that of the serum added. Ultimate dilutions are the ones tabulated. In Table 4 the results of tests with serums of various apparently normal individuals and various strains of Streptococcus salivarius are given.

TABLE 4

AGGLUTINATION OF S. SALIVARIUS BY NORMAL SERUM

Organism	Serum	Dilutions						
		1:10	1:20	1:40	1:60	1:100	1:150	1:200
C	A	++++	++++	++++	++++	++	--	—
C	D	++++	++	—	—	—	—	—
F	I	—	—	—	—	—	—	—
F	E	++++	++++	+	—	—	—	—
C	C	+++	++	+	—	—	—	—
A	A	++	—	—	—	—	—	—
F	K	—	—	—	—	—	—	—
F	B	—	—	—	—	—	—	—
F	A	++++	++++	++++	+++	—	—	—
H	A	—	—	—	—	—	—	—
C	B	—	—	—	—	—	—	—
C	I	—	—	—	—	—	—	—
F	F	+	—	—	—	—	—	—

It is interesting that among pleomorphic streptococci from over 300 throat cultures there was not a single strain of Strep. mitis. The

only mitis strain was obtained from a head wound in a boy Table 5 gives the results of agglutination tests with normal human serum and this organism.

TABLE 5

AGGLUTINATION REACTIONS OF S. MITIS WITH NORMAL SERUM

Serum	Dilutions						
	1:10	1:20	1:40	1:60	1:100	1:150	1:200
I	+++	++++	+++	+++	+++	—	—
A	+++	++	—	—	—	—	—
D	—	—	—	—	—	—	—
C	++	++	—	+++	+++	—	—
B	—	—	—	—	—	—	—
E	—	—	—	—	—	—	—
G	+	—	—	—	—	—	—

We see that normal agglutinins for S. salivarius and S. mitis were present in some individuals as high as 1 : 100.

A rabbit was immunized against one strain of S. salivarius and complement fixation tests made with various streptococci. Serum from A, C, and D, 3 normal persons, whose blood possessed agglutinins was tested at the same time. Table 6 gives the results.

TABLE 6

COMPLEMENT FIXATION

Antigen	Serum			
	A	D	C	Immune Rabbit Serum
C	—	—	—	++++
G (Strep. mitis)	—	—	++++	++++
F	—	—	—	++++
Strep. pyogenes	—	++++	—	—
Strep. viridans	—	—	—	—
A	—	—	—	++++

According to the complement fixation tests S. salivarius and the one strain of S. mitis acted alike, and were sharply differentiated from the strains of St. pyogenes and S. viridans.* We found only occasional complement fixation bodies for streptococci in normal serum.

The results of tests to show whether rabbits inoculated with pleomorphic streptococci develop agglutinins are given in Table 7.

Before inoculation the blood of the rabbits was tested several times for normal agglutinations, but none were observed. We note that

* The organism was isolated from the blood stream of a patient with subacute endocarditis. On plain blood agar it produces small, nonhemolytic, decidedly green colonies. Morphologically it is not pleomorphic but possesses from 2-6 very small and uniform cells to each chain. Culturally, according to Holman, it is S. salivarius.

agglutinins against the strains used developed to 1:20 in one animal and 1:50 in the others. Other strains of S. salivarius were agglutinated by dilutions of 1:100.

Table 8 gives the results of agglutination tests of various strains of streptococci with Rosenow's antipolimyelitis serum.

TABLE 7

AGGLUTINATION OF S. SALIVARIUS BY SERUM FROM INOCULATED RABBITS

Organism	Method of Inoculation	Dosage, C C	Condition of Animal	Organism	Results					
					1:10	1:20	1:50	1:100	1:200	1:400
F	Intravenous	10	Rise of temperature after 12 days	F	+++	++				
				L	++++	++++	++++	++++	—	—
2-2	Intracerebral	0.5	Permanent impairment of both hind legs	F	+++	++	—	—	—	
				L	++++	+++	+++	++	—	—
				2-2	++++	+++	++			

TABLE 8

AGGLUTINATION WITH ANTIPOLIOMYELITIS SERUM

Organism	Antipoliomyelitis Serum								
	1:25	1:50	1:100	1:200	1:500	1:1,000	1:1,500	1:2,000	1:3,000
F	++++	++++	++++	++++	++++	++++	+++	—	—
C	++++	++++	++++	+++	—	—	—	—	—
G	+	—	—	—	—	—	—	—	—
Strep. mitis	++++	+	—	—	—	—	—	—	—
1016	+++	++	++	—	—	—	—	—	—
1007	—	—	—	—	—	—	—	—	—
	++++	+++	+++	++++	++	—	—	—	—
L	++++	++++	++++	—	—	—	—	—	—
11-1	++	+	++	—	—	—	—	—	—
H	—	—	—	—	—	—	—	—	—
A	+	—	—	—	—	—	—	—	—
Strep. fecalis	—	+	+	—	—	—	—	—	—
Strep. pyogenes	+	+	..	—	—	—	—	—	—
Strep. viridans	—	—	—	—	—	—	—	—	—

It will be observed that antipoliomyelitis serum agglutinates strains of S. salivarius quite uniformly, but in variable dilutions. Strain F, for example, was agglutinated in dilutions of 1:1,500, whereas Strain C was agglutinated only by 1:200, and one strain of S. mitis was clumped by 1:50 dilution. All 3 of these strains showed ability to localize in the nervous system of young rabbits and produce changes accompanied by loss of muscle tone and rise in temperature. Strain C was more virulent for young rabbits than Strain F. The culture of S. mitis (G) was also more virulent than Strain F of salivarius.

DISCUSSION

In the experiments by Rosenow and his associates,[2] 85% of the animals showed loss of muscle tone and 15% flaccid paralysis. In our experiments 54% showed loss of muscle tone, 4.55% flaccid paralysis, and 22% rise in temperature only. The changes were increase in neuroglia and round cells and perivascular infiltration in the brain and cord. They mention that the streptococci lost their virulence rapidly and unless very early transplants were used the results were negative. We found this to be only partially true, as some of our highly virulent cultures gave positive results even after 12 weeks, being carried along on fresh serum agar and transplanted every 2 or 3 days. We found ascitic fluid dextrose broth to be very satisfactory, but also obtained positive results from dextrose serum agar plates. Our results seem to confirm most of the observations by Rosenow and his co-workers. However, there seems to be a difference of opinion as to the interpretation of the results. Bull[5] occasionally obtained flaccid paralysis in rabbits with streptococci, and concluded that this depended on the virulence of the organism used, rather than the source. Negative results on inoculation of rabbits with filtrates of extracts of the brain and cord of poliomyelitis patients are explained by Rosenow as probably due to adult rather than young rabbits having been used. He, however, has not been successful in producing poliomyelitis in monkeys with filtrates of pleomorphic streptococci, and this is attributed to imperfect technic. Rosenow bases his conclusions that poliomyelitis is caused by a pleomorphic streptococcus on the following points: The streptococcus was found uniformly in poliomyelitis, especially the central nervous system and the throat; it produces loss of muscle tone, flaccid paralysis, rise in temperature, in animals, with round cell infiltration in the central nervous system; the blood of poliomyelitis patients contains agglutinins for this streptococcus; and specific immune serum seemed to cause an initial rise followed by a marked drop in temperature and improvement in the symptoms of poliomyelitis patients.

In our work we have found that pleomorphic streptococci, culturally and morphologically similar to the organisms found in poliomyelitis, have a wide distribution, being found in 20-25% of the throats of normal persons not associated with any outbreak of poliomyelitis; that these organisms are capable of producing similar conditions in

[5] Jour. Exp. Med., 1917, 25, p. 557.

rabbits as those from poliomyelitis and apparently have a predilection for the central nervous system. Previously one of us[1] reported on what seemed like marked beneficial action of diphtheria antitoxin in tonsillitis in which pleomorphic streptococci appeared to be the etiologic factor. Following the injection there was usually an initial rise in temperature succeeded by a drop and marked improvement. These results were attributed to nonspecific protein effects. Rosenow,[6] however, reports such striking results with antistreptococcus serum in poliomyelitis that it is difficult to exclude specific action.

Rosenow, Towne and Hess state that in experiments with many strains of streptococci other than the pleomorphic they have not been able to produce the poliomyelitis-like symptoms and the peculiar localization which they did with the latter organisms. We hope to study these questions further; at present we can say that one strain of non-pleomorphic Streptococcus salivarius showed the same tendency to remain localized in the central nervous system, but it only produced general weakness and rise in temperature, with no indication of loss of muscle tone or flaccid paralysis.

Since Rosenow's early work on elective localization there have been published numerous articles, some of which seemed to confirm and others to disprove his contentions. Gay[7] does not conclude that Rosenow's results have been discredited and thus his positive results should take precedence over negative results by others.

In the work of Moody[8] and of Detweiler and Maitland[9] no attention was paid to the particular strains of streptococci other than to use organisms that might be called members of the viridans group. Krumweide and Valentine[10] have shown that the viridans group is a decidedly heterogeneous one.

Henrici's[11] work, however, was planned with the aim of comparing the tendency of specific organisms to localize. So also was the work of Rothschild and Thalhimer[12] on S. mitis. Unfortunately, in Henrici's work the central nervous system was not examined thoroughly, but he mentions certain interesting nervous systems. He concludes that fermentation tests are of no significance from the standpoint of virulence

[6] Jour. Infect. Dis., 1918, 22, p. 379.
[7] Jour. Lab. and Clin. Med., 1918, 3, p. 721.
[8] Jour. Infect. Dis., 1916, 19, p. 515.
[9] Jour. Exper. Med., 1918, 27, p. 37.
[10] Jour. Infect. Dis., 1916, 19, p. 760.
[11] Jour. Infect. Dis., 1916, 19, p. 572.
[12] Jour. Exper. Med., 1913, 19, p. 429.

or tissue localization. The work of Thalhimer and Rothschild was confined to a study of involvement of the heart by S. mitis.

Detweiler and Maitland[9] apparently assume that it is unnecessary to try to classify the various streptococci used other than to group them as S. viridans. They cite Henrici's results in justification, but in view of Henrici's failure to include data on localization in the nervous system it is perhaps unwarranted to make such comparisons.

In regard to the tendency to localization in the nervous system observed by us, we venture to suggest that these strains of S. salivarius are not highly virulent in that they do not possess marked invasive powers; when injected into the blood stream in sufficiently large doses, the mechanisms for ridding the blood stream of bacteria is not able to remove all before some reach the nervous system. When once in the nervous system in sufficient number the organisms multiply because of small tendency for phagocytosis by fixed tissue cells, because the cellular reaction is monunclear rather than polymorphonuclear and hence little phagocytosis occurs from mobile cells, and further because the medium is favorable for growth.

This explanation is bourne out by the fact that when injected directly into the brain tissue the organisms do not invade the general circulation but spread throughout the nervous system only. It is possible that they are taken into the general circulation, but if they are they seem to be rapidly disposed of by phagocytic cells and were not found except when the animal succumbs to overwhelming doses within a few hours after injection.

We do not mean to say that no strains of S. salivarius will localize elsewhere than in the nervous system and occasionally in the heart; we only say that of the animals examined 48 hours or more after inoculation all showed localization in the nervous system and one also in the myocardium.

It is possible that there is a direct relationship between the globoid organisms described by Flexner and Noguchi[13] in poliomyelitis and pleomorphic streptococci. If so it would be of interest that organisms with similar effects on rabbits are so widely distributed as indicated by our results.

SUMMARY

We found pleomorphic streptococci which according to Holman would be classed as S. salivarius in the nasopharynx of approximately 25% of apparently normal persons.

[13] Jour. Exper. Med., 1912, 18, p. 411.

These organisms as a rule showed some pathogenicity for young rabbits, which develop agglutinins for the strains of organisms injected.

Injected intracerebrally, the cocci remained localized in the nervous system throughout which they became disseminated.

When injected intravenously they were found in the nervous system within 3 or 4 hours. After 72 hours they were present usually only in the nervous system.

The common symptoms were rise in temperature and loss of muscle tone, with occasional flaccid paralysis and permanent impairment of the legs.

The changes in the nervous system were hyperemia, frequently hemorrhage, increase in round and neuroglia cells and perivascular infiltration of round cells.

The serum of normal persons frequently contains agglutinins for these organisms, occasionally in dilutions of 1 : 100.

The agglutinin titer of the serum of horses which Rosenow injected with streptococci from poliomyelitis was found to be the same for the streptococci we studied as for the poliomyelitis cocci.

EXPLANATION OF PLATE

Fig. 1.—Loss of muscular power. Received 0.5 c c of a twenty-four-hour culture intracerebrally.

Fig. 2.—Marked loss of muscular power lasting more than 3 weeks.

Fig. 3.—Received 10 c c of a twenty-four hour culture intravenously; marked loss of muscle power. Permanent impairment with muscular atrophy of hind legs.

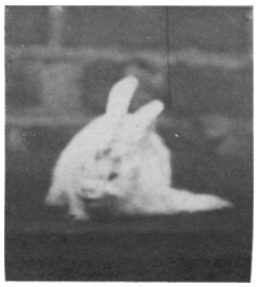
Fig. 4.—Cell infiltration near point of inoculation in posterior part of brain.

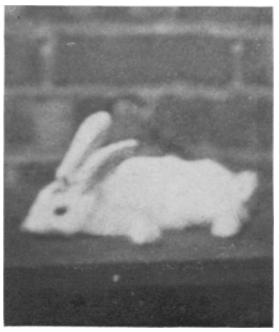
Figs. 5 and 6.—Streptococcus salivarius showing some of the pleomorphic characteristics.

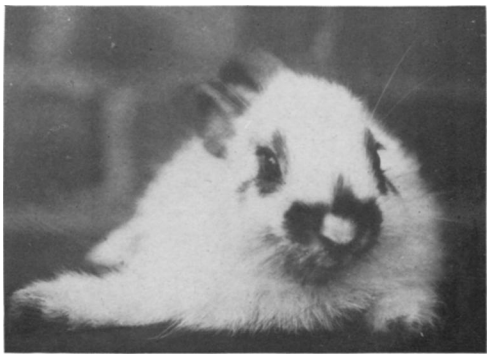
Fig. 7.—Perivascular infiltration in anterior part of brain, point of inoculation being in posterior part.

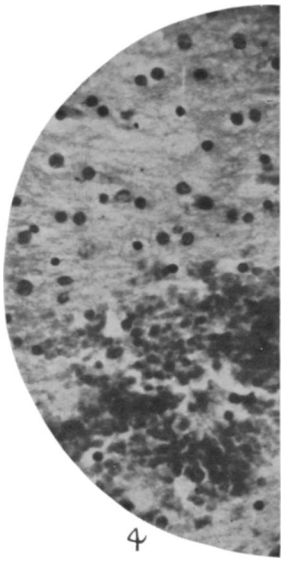
Fig. 8.—Round cell infiltration in posterior part of cerebrum following intravenous inoculation.

Plate









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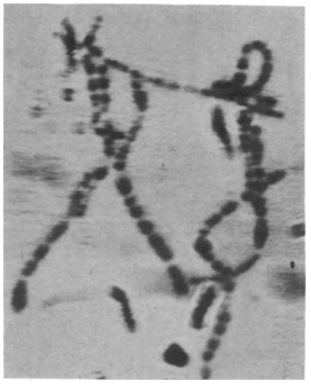

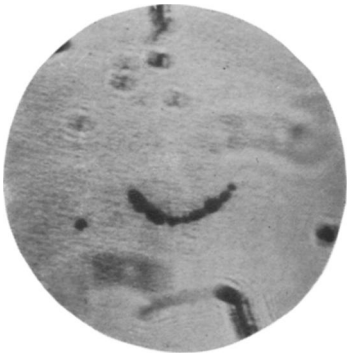

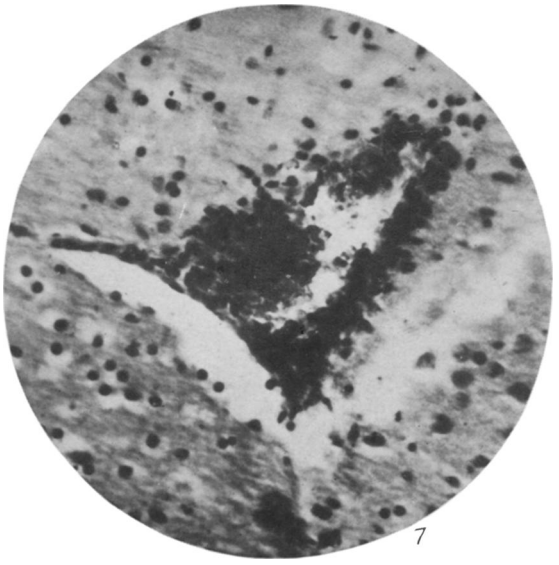

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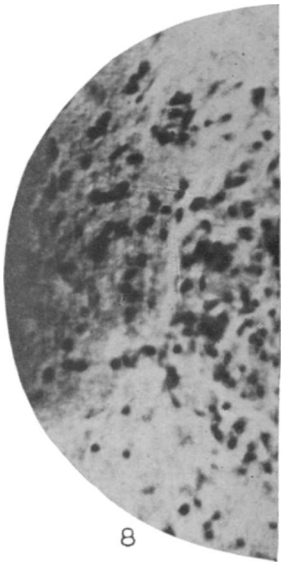

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